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THE HYDROLYSIS OF SUCROSE BY INVERTASE AT HIGH SUGAR CONCENTRATIONS

DISSERTATION

Submitted in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy in the Faculty of Pure Science at Columbia University.

BY

CHANDLER D. INGERSOLL, B.S., M.A.

New York City
1925

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Dedicated
to my
Father and Mother

ACKNOWLEDGMENT

The author wishes to express his most sincere appreciation to Professor John M. Nelson, at whose suggestion the following investigation was undertaken, for his constant interest, helpfulness, and co-operation throughout this work.

PART I.

THE EFFECT OF SUCROSE CONCENTRATION ON THE VELOCITY OF HYDROLYSIS

Brown[1] showed that the intitial rate of hydrolysis of sucrose solutions by invertase increased with increasing sucrose concentrations up to a point, which Michaelis and Menton, Nelson and Vosburgh, and Euler and Myrbaeck have shown to represent a sucrose concentration of about 5 per cent,* but at sucrose concentrations above 5 per cent seemed to remain constant. Michaelis and Menton[2] however, in working with sucrose concentrations up to .77 normal noted that this maximum did not maintain itself but that on further increasing the concentration of sucrose the initial velocity of hydrolysis decreased. Their hypothesis had required that this velocity should assymptotically approach a maximum. In commenting on the probable causes of this phenomenon, these investigators said,—

> "The most important influence is what might be summarized as the 'change in nature of the solution medium.' In a normal, that is to say 34 per cent sugar solution, we may no longer consider the water simply as solution medium. Further the affinity constant of ferment for sugar is changed and perhaps the velocity constant for the breaking-up of the compound (invertase-sucrose compound) will also be different."

Euler and Myrbaeck[3] have also studied the velocities of hydrolysis in the case of higher sucrose concentrations and claimed that the velocity remained constant for wider range of sucrose concentrations, i.e., between 5 per cent and 20 per cent, than was previously stated by Michaelis and Menton, but that beyond 20 per cent they also found that the velocity decreased. Their explanation for this decrease was similar to the one offered by Michaelis and Menton.

Nelson and Vosburgh[4] had however previously investigated sucrose solutions up to 20 per cent concentration and found no such constancy of velocity as reported by Euler

*NOTE:—The term *per cent* is used in this paper to signify grams of sugar per 100 cc of solution.

and Myrbaeck. Their results showed that a maximum velocity was attained at a sucrose concentration of about 5 grams per 100 cc of solution, but that a concentration of 20 grams per 100 cc of solution this maximum velocity had fallen off considerably. They are thus in accord with Michaelis and Menton.

Colin and Chaudun[5] have been the only investigators that have offered a specific explanation of this decrease in velocity at high sucrose concentrations and claim to have shown that the decreasing velocity can be accounted for by the increasing viscosity of the sucrose solutions. They formulated this relation as a general law of enzyme hydrolysis.

REASONS FOR THE PRESENT INVESTIGATION

With the exception of Colin and Chaudun and of Euler and Myrbaeck, all investigators studying the kinetics of the invertase hydrolysis have limited their investigations to solutions of relatively low sucrose concentrations. The main reasons for this are the experimental difficulties encountered in working with the viscous solutions met with at high sucrose concentrations. These difficulties concern themselves with such items as the volumetric pipetting of hydrolysis samples, and the starting and stopping of the hydrolysis at a definite time by the uniform introductions into the viscous sucrose solutions of the invertase and sodium carbonate* solutions, respectively. In brief, the three main errors that would be encountered and their effects on velocity measurements of the hydrolysis are:—

1. The inability to distribute the invertase solution uniformly through the sucrose-buffer solution would cause the hydrolysis mixture to be heterogeneous with respect to water, and the invertase (being in the more dilute portions of the hydrolysis mixture) would hydrolyze the sucrose at a more rapid rate than would be representative for a given sucrose concentration. The velocities obtained would in this case be too high.

*NOTE:—M/5 sodium carbonate solution was the alkaline reagent here used to stop the hydrolysis when samples were taken.

2. The measuring out of the volume of samples, by which to determine the comparative velocities of hydrolysis of different sucrose concentrations, by a pipette would give volumes that would be too small for the higher sucrose concentrations. As the volume of sodium carbonate solution added in each case is constant, the rotation readings for the higher sucrose concentrations would progressively be too small as the sucrose concentration of the hydrolysis mixture was increased. Due to this error the hydrolysis velocities for the more concentrated sucrose solutions would be too small to be comparable to the velocities obtained with more dilute sucrose solutions.

3. The inability to distribute the sodium carbonate solution uniformly through the hydrolyzing mixture at definite time would result in hydrolysis beyond the time noted for stopping the reaction and this condition would be more pronounced the higher the sucrose concentration. Due to this error the velocities obtained for the more concentrated sucrose solutions would be too high.

The total result of the three above-mentioned errors would be to give higher velocities than should be obtained for a hydrolyzing mixture of high sucrose concentration. In figure 7 may be seen a comparison of the observed velocities in this investigation with those obtained by Colin and Chaudun and by Euler and Myrbaeck.

SYNOPSIS OF THE PRESENT INVESTIGATION

Due to some modifications in procedure in mixing the solutions of sucrose and invertase and in measuring the samples by weighing instead of pipetting, the velocity of hydrolysis for higher sucrose concentrations has been studied again and the present paper is an account of the results obtained.

Solutions varying in sucrose concentration from 3½ per cent to 69 per cent were hydrolyzed by means of invertase

and the procedure adopted was such as to make it possible to study the influence of water, as well as that of sucrose, on the velocity of hydrolysis.

We here mean the term velocity in the same sense that it has been employed by Michaelis and Menton,[2]—that is, to be represented by the change in rotation per unit time of the hyrdolysis mixture, or stated another way, to be the number of grams of sucrose hydrolyzed per unit time. Velocity of hydrolysis is the customary method of measuring the comparative activity of the enzyme.

The retardant action of invert sugar and that of a-methyl glucoside have been studied over a wider range of sugar concentrations than has been done heretofore. In the case of invert sugar this has been done by three series of experiments in which, for each series, the same fraction of the total sugar in solution was present as invert sugar, and by a fourth series in which the amount of invert sugar added was in each case constant. The effect of the a-methyl glucoside was studied over a similar range of sucrose concentrations using a constant amount of glucoside in each case.

Besides this the influence of hydrogen ion concentration was studied in order to see whether or not the hydrogen ion concentration of the hydrolysis mixture would affect the velocity of hydrolysis in the same manner at high sucrose concentrations that it does at low concentrations. The sucrose concentrations investigated were 10 per cent, 35 per cent, and 57 per cent, while the range of hydrogen ion concentration was from PH 3.0 to PH 7.5.

THE METHOD USED IN THE PRESENT INVESTIGATION

The method of procedure devised and adopted for the purpose of the present investigation was as follows:—

The sugar was weighed out and transferred into wide-mouthed hydrolysis bottles containing the buffer-water solution. After the sugar had dissolved these solutions were allowed to stand in the thermostat, as was also the inver-

tase solution, at least an hour before the hydrolysis was started in order to bring them to 25° C. temperature.

The solution was then agitated by an electrically-driven glass stirring paddle and the invertase introduced by means of a pipette. The paddle so rotated as to immediately pull the lighter invertase solution down into the heavier sugar solution and forcibly throw it against the sides and the bottom of the hydrolysis bottle. In this way the invertase was quickly distributed throughout the solution. The starting time was taken to be the time the pipette started to flow plus half the time of flow of the pipette. The error so introduced would in all cases be exceedingly small and in the same direction. It might vary slightly in magnitude with changing sucrose concentrations.

The 25 cc samples were taken by weight. On the basis of specific gravities, previously determined by means of a 25 cc (approx) picnometer in the usual manner, counterweights were made to exactly represent 25 cc of the different invertase sugar solutions at 25° C. Sample bottles were then tared on a balance having an accuracy of 0.01 gram and, by means of these counterweights, a weight of solution equivalent in volume to 25 cc of the hydrolyzing mixture* was pipetted into the sample bottles. All the samples required, to follow the course of the hydrolysis at certain given time intervals, were weighed out immediately after the reaction had been started, the bottles containing the samples placed in the bath, and the hydrolysis allowed to proceed to the expiration of the given time intervals. The evident source of error in this procedure was the cooling of the hydrolysis mixture during the time of weighing. By considerable experimental practice this time was reduced to about one minute and, since the room temperature was not very different from that of the thermostat, the cooling during this interval was considered negligible.

When stated time intervals had elapsed the hydrolysis

*NOTE:—The terms "hydrolysis solution" and "hydrolysis mixture" are used synonymously throughout this paper.

was stopped by adding 10 cc of M/5 sodium carbonate solution to the 25 cc of the hydrolyzing solution contained in the sample bottle. The error here, due to the ability to uniformly introduce the carbonate solution into the viscous hydrolysis mixture at a definite time, was taken care of by the use of a relatively large (in comparison with ordinary procedure) volume of carbonate solution and continuous agitation by hand of the bottle containing the sample during the time of flow of the pipette. The time of stopping was computed in the same manner as the time of starting.

All of the above errors were extremely small and duplicate hydrolyses of the same mixture showed that any effect due to them was so constant that it was undetectable on the polariscope.

In the case of mixtures having a sugar concentration less than 12 grams to 14 grams per 100 cc of solution, the usual and much simpler procedure[6] of measuring out the hydrolysis samples by volume was employed. It was found experimentally that the error so introduced in determining the initial velocity was undeterminable.

THE RELATION BETWEEN THE VELOCITY OF HYDROLYSIS AND VARYING SUCROSE CONCENTRATION

Various amounts of sucrose were transferred into hydrolysis bottles containing 200 cc of M/20 secondary sodium citrate buffer (to give a PH in the neighborhood of 5.00) and the sucrose allowed to dissolve in it. When these sucrose solutions had come to proper temperature in the thermostat (25°) the reaction was started in each case by the introduction of 25 cc of invertase solution in the manner noted above.

The work was carried out with two different preparations of yeast invertase in order to show that the effects noted were independent of the method of preparation. Invertase A-1 was prepared by the alcohol-precipitation method as given by Nelson and Hollander[7] and was the

same as Invertase No. 16 of Washburn[8] diluted 75 times. Invertase B was prepared by the lead-acetate method of Nelson and Born[9] and was the same as Invertase No. 4 of Washburn,[8] diluted 3 times.

The temperature of the hydrolyses was maintained constant at 25° C. by means of a thermostat regulated according to the method of D. J. and J. J. Beaver.[10]

The PH of the mixtures was determined by the hydrogen-electrode E. M. F. method and varied slightly as between different concentrations of sucrose. This is treated more fully on P. 22.

The course of the hydrolysis was followed polarimetrically, the temperature of the polariscope tubes while reading being maintained at 25° according to the method of Beegle.[11] The length of the polariscope tubes used throughout this investigation was 200 mm. The use of the polariscope at higher sucrose concentrations is justified on P. 20.

Tables 1, 2 and 3 show the results obtained.

TABLE I

COMPOSITION OF SOLUTIONS

A constant amount of water (225 cc) was used with each hydrolysis mixture, being added in the form of:—

M/20 Secondary Sodium Citrate Buffer Solution 200 cc or 200.976 gms
Invertase 25 cc or 24.926 gms.

Mixture	Sucrose added gms	Specific Gravity gms/cc	Total Solution Volume cc	Sucrose Concent %	Sucrose Concent M/L*	Water Concent M/L*
a	8.0	1.01670	230.05	3.478	0.10161	54.015
b	12.5	1.02623	232.85	5.369	0.15686	53.368
A	20.0	1.03709	237.11	8.435	0.24647	52.409
AA	35.0	1.05988	246.16	14.218	0.41546	50.481
B	50.0	1.08021	255.42	19.575	0.57199	48.651
BB	75.0	1.11054	270.95	27.680	0.80880	45.862
C	100.0	1.13793	286.39	34.917	1.02026	43.389
CC	150.0	1.18143	318.17	47.144	1.37753	39.056
D	200.0	1.21806	349.65	57.198	1.67131	35.538
E	275.0	1.25936	397.74	69.140	2.02230	31.242

*Note:—The abbreviation M/L signifies "Moles per Liter."

TABLE II. SECTION A.

INDIVIDUAL HYDROLYSES USING INVERTASE A-1

The initial rotations of the various hydrolysis mixtures in this and the following tables are obtained through extrapolation. The method of extrapolating these values and its justification is explained on page 19.

Two different batches of Invertase A-1 were used in the hydrolyses recorded here. This is taken up more fully at the head of section B of this table.

Temperature 25° C. PH 5.00

Mixture a			Mixture b		
Time Minutes	Rotation*	Change	Time Minutes	Rotation*	Change
00	(3.91)	—	00	(5.98)	—
05	3.74	.17	10	5.63	.35
10	3.57	.34	20	5.27	.71
20	3.24	.67	30	4.93	1.05
30	2.93	.98	60	3.96	2.02

Mixture A			Mixture AA		
Time Minutes	Rotation*	Change	Time Minutes	Rotation*	Change
00	(9.37)	—	00	(15.77)	—
10	9.04	.33	10	15.48	.29
20	8.72	.65	30	14.91	.86
30	8.37	1.00	60	14.05	1.72
70	7.07	2.30	90	13.19	2.58

Mixture B			Mixture BB		
Time Minutes	Rotation*	Change	Time Minutes	Rotation*	Change
00	(21.77)	—	00	(30.84)	—
30	21.04	.73	30	30.25	.59
60	20.33	1.44	60	29.64	1.20
120	18.86	2.91	120	28.44	2.40
240	16.01	5.76	240	26.03	4.81

Mixture C			Mixture CC		
Time Minutes	Rotation*	Change	Time Minutes	Rotation*	Change
00	(38.85)	—	00	(52.38)	—
30	38.33	.52	30	51.99	.39
60	37.82	1.03	60	51.62	.76
120	36.77	2.08	120	50.82	1.56
240	34.73	4.12	240	49.38	3.00

TABLE II. SECTION A—*Continued.*

Mixture D			Mixture E		
Time Minutes	Rotation*	Change	Time Minutes	Rotation*	Change
00	(63.79)	—	00	(77.02)	—
30	63.54	.25	60	76.73	.29
60	63.29	.50	120	76.44	.58
120	62.79	1.00	180	76.15	.87
240	61.81	1.98			

*For "absolute rotations," see page 25.

TABLE II. SECTION B.

INITIAL VELOCITIES USING INVERTASE A-1

The hydrolyses of Mixtures B, BB, C, D, and E as given above were made with a first batch of Invertase A-1, while a second batch of the same invertase was used for the remaining hydrolyses. Since it has been shown by Nelson and Vosburgh[1] that the rate of hydrolysis is directly proportional to the concentration of invertase in the mixture, this second batch of invertase was standardized against the first batch and its activity found to be greater with a conversion factor of X .933.

Mixture	Observed Velocity °/hr.	Invertase Factor	Velocities Corresponding to use of first batch of Invertase A1	Corrected Velocity °/hr.
a	2.01	.933	1.88	1.92
b	2.10	.933	1.95	2.02
A	2.00	.933	1.86	1.96
AA	1.72	.933	1.60	1.75
B	1.45	1.000	1.45	1.65
BB	1.20	1.000	1.20	1.46
C	1.03	1.000	1.03	1.31
CC	.77	.933	.72	1.02
D	.50	1.000	.50	.78
E	.29	1.000	.29	.51

TABLE III. SECTION A.

INDIVIDUAL HYDROLYSES USING INVERTASE B

Temperature 25° C. PH 5.00

Mixture a

Time Minutes	Rotation*	Change
00	(3.83)	—
05	3.33	.50
10	2.85	.98
15	2.39	1.44
20	1.95	1.88

Mixture b

Time Minutes	Rotation*	Change
00	(5.92)	—
05	5.42	.50
10	4.91	1.01
20	3.90	2.02
30	2.99	2.93

Mixture A

Time Minutes	Rotation*	Change
00	(9.28)	—
05	8.79	.49
10	8.28	1.00
20	7.30	1.98
30	6.33	2.95

Mixture B

Time Minutes	Rotation*	Change
00	(21.71)	—
10	20.92	.79
20	20.13	1.58
30	19.33	2.38
60	16.96	4.75

Mixture C

Time Minutes	Rotation*	Change
00	(38.70)	—
10	38.17	.53
20	37.61	1.09
30	37.06	1.64
60	35.42	3.28

Mixture D

Time Minutes	Rotation*	Change
00	(63.39)	—
30	62.56	.83
60	61.79	1.60
90	61.01	2.38
120	60.19	3.20

Mixture E

Time Minutes	Rotation*	Change
00	(76.62)	—
30	76.17	.45
60	75.70	.92
90	75.22	1.40
120	74.79	1.83

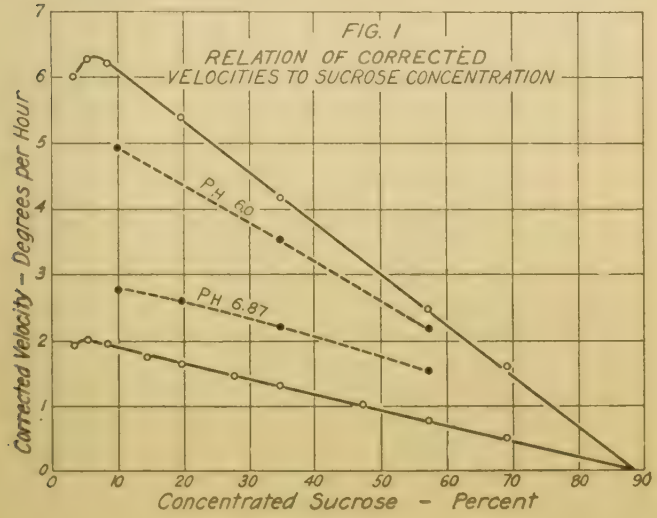
*For the "absolute rotations," see page 25.

TABLE III. SECTION B.
INITIAL VELOCITIES USING INVERTASE B.

Mixture	Observed Velocity °/hr.	Corrected Velocity °/hr.
a	5.88	6.01
b	6.06	6.27
A	5.90	6.22
B	4.76	5.40
C	3.28	4.17
D	1.60	2.49
E	.91	1.61

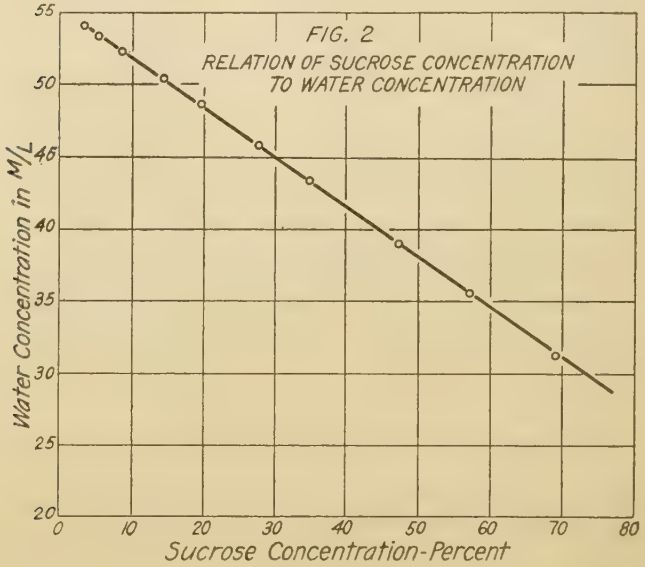
THE CORRECTED VELOCITIES

It will be noticed in tables 2 and 3 above that we have tabulated a "corrected velocity" to correspond to each observed velocity. It has been the aim of this investigation to ascertain as far as possible the effect on the velocity of reaction of an increase in sucrose concentration alone. Therefore it becomes necessary to consider velocities that

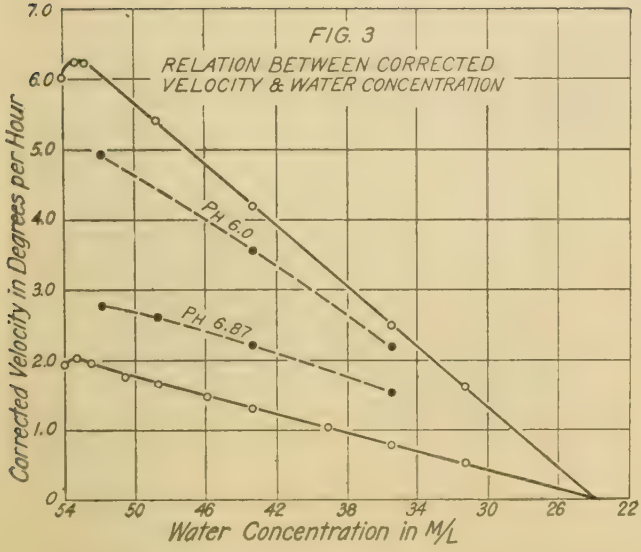


would represent hydrolysis mixtures in which all the components except sucrose were held constant.

Thus we might measure the change in rotation for equal quantities of water and invertase by employing polariscope tubes of such various lengths for the different sucrose con-



centrations that the rotation measurements would in all cases be for the same amounts of water and invertase. This method would however be impracticable and the same result may be accomplished by considering the changes in volume of solution that result when different amounts of sugar are added to equal quantities of water (equal quantities of water have been employed in each hydrolysis mixture) and by multiplying the changes in rotation observed on the polariscope, which are for the same absolute quantities of solution, by a volume factor,—the volume of solution divided by the volume of water present (added).



Thus are obtained the "corrected velocities," by which are meant the changes in rotation that take place in the same alliquot portion of each hydrolysis mixture.

DISCUSSION

It may be seen from figure 1 that when these corrected velocities are plotted against their respective sucrose concentrations that a linear function is apparently obtained with each invertase preparation. This would seem to indicate that, by using the corrected velocities, the velocity of hydrolysis may be expressed as a simple inverse linear function of the sugar concentration.

It is a coincidence, however, that the water concentration of sucrose solutions is also practically an inverse linear function of the sucrose concentration (fig. 2) and thus we likewise obtain a linear relationship between the corrected

velocities and the respective water concentrations. This is shown in figure 3. It might here be pointed out that the volume correction noted above would be the same numerical one that we should apply if we were to consider that the hydrolysis took place only in the aqueous portion of the hydrolysis mixture,—i.e., the water would be the limiting factor and we should consider the comparative reaction volume in each case to be equal quantities of water.

Whether it is the water that is the limiting factor of the hydrolysis at high sucrose concentrations or whether it is the sucrose, it is thus impossible to say.

If, for different sucrose concentrations (and water concentrations) we now plot such ideal velocities for hydrolyses in which the hydrogen-ion concentration is not in the immediate region of the optimum, these apparent linear relationships between velocity and concentration take on a slight curvature in each case. In table 4 below are given the initial velocities for three different sucrose concentrations as read off the PH-velocity curves, figure 11, for PH's 6.00 and 6.87 together with the corresponding corrected velocities. These values are plotted in figures 1 and 3 and connected by dotted-line curves. The point representative of mixture B was determined by experiment.

TABLE IV.

INITIAL AND CORRECTED VELOCITIES FOR PH 6.00 AND PH 6.87

	PH 6.000		PH 6.87	
Mixture	Observed	Corrected	Observed	Corrected
10	4.62	4.92	2.60	2.77
B	—	—	2.30*	2.61
C	2.78	3.54	1.74	2.21
D	1.41	2.19	.98	1.52

*Experimental Observation.

Thus it is seen that these linear velocity relationships mentioned above only hold rigidly in the neighborhood of the PH of maximum velocity.

THE DETERMINATION OF INITIAL AND FINAL ROTATIONS

The initial rotations given in all tables in this paper are extrapolated values. This has been signified by enclosing the figures for these initial rotations in parentheses. In consideration of this and of the fact that the interests of this paper are concerned with the changes in rotation rather than the rotations themselves, the rotations given in the hydrolysis tables throughout have not been corrected for the small rotations of the two invertases used:—

Rotation of 25 cc of Invertase A-1 in 200 cc of Buffer, 25° = +.005
Rotation of 25 cc of Invertase B in 200 cc of Buffer, 25° = +.08

or for the small zero errors of the polariscope which remained constant for the individual determinations and did not vary in any instance from the scale zero by more than .05 of a degree.

The extrapolation of the initial rotations was made by adding to the rotation of the first sample taken for each hydrolysis, such an amount that would represent the fractional part of a degree that the rotation of the hydrolysis mixture would have changed between the time the hydrolysis was started and the time the sample was taken. This fractional part of a degree was taken to be the same as the observed change in rotation at the start of the hydrolysis (during the period when the change in rotation per unit time remained constant within experimental error) for a length of time equal to that between the time of starting and of taking the first sample. The extrapolated values for the initial rotations given in table 5 below are those as given in table 2A corrected for the polariscope error. The correction for the rotation of the invertase A-1 was considered too small to be applied.

In order to determine whether such extrapolations were permissible, a series of rotation determinations were made on sucrose solutions containing no invertase and of the same concentrations as were used in the course of this in-

vestigation. By reference to table 5 it can be seen that the use of extrapolated initial rotations is justifiable.

A corresponding series of determinations was made for the final rotations of the completely hydrolyzed mixtures. In one case the rotations represent those obtained from actual hydrolysis of the sucrose solutions and in the other those obtained from solutions containing invert sugar and water equivalent in amount to compositions corresponding to complete hydrolysis of the various mixtures. A stronger invertase was used for completely hydrolyzing the more concentrated sucrose solutions.

These initial and final rotations are given in tabular form below and in figure 4 are plotted against the respective concentrations.

TABLE V.

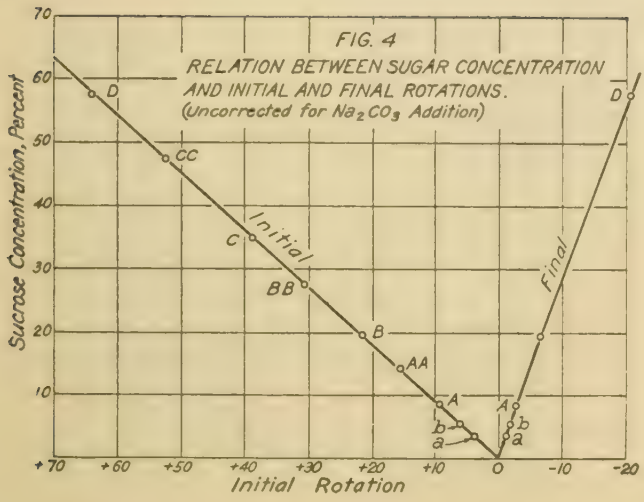
INITIAL AND FINAL ROTATIONS.

Mixture	Extrapolated Initial Rotation*	Rotation of Sucrose Solutions of equal concentration in the absence of Invertase.	Observed Final Rotations of the Hydrolysis Mixtures	Rotations of Invert Sugar Solutions Representing Complete Hydrolysis
A	9.38	.939	–2.78	—
AA	15.79	15.80	–4.70	—
B	21.77	21.76	–6.70	–6.71
BB	30.82	30.81	—	—
C	38.82	38.80	–12.19	–12.29
CC	52.38	52.38	—	—
D	63.79	63.80	–20.85	–20.85
E	77.00	76.99	—	—

*For "Absolute Rotations." See page 25.

THE USE OF THE POLARISCOPE IN THE ANALYSIS OF CONCENTRATED SUGAR SOLUTIONS

Rosanoff[12] investigated the use of the polariscope for a sucrose solution of approximately 20 per cent concentration and said that at this concentration the polariscope



could be used to show the composition of the solution. Vosburgh[13] claimed that the polariscope gives slightly high values for the amount of sucrose hydrolyzed. To see if the use of the polariscope were permissible to follow the course of hydrolysis in high sucrose concentrations, rotations were obtained for solutions, representing mixture C and D, in which 20 per cent, 40 per cent, and 60 per cent of the sucrose was replaced by invert sugar. Allowance was made for the amount of water that would have been used up in the course of the hydrolysis and so represented in the invert sugar. These values, given below in table 6, were plotted against Per Cent Hydrolysis in figure 5 and a straight line obtained in both cases.

On the basis of this, points taken from the complete hydrolysis figures (table 13) for these mixtures were also plotted and proved to be in good agreement with the respective straight lines mentioned above.

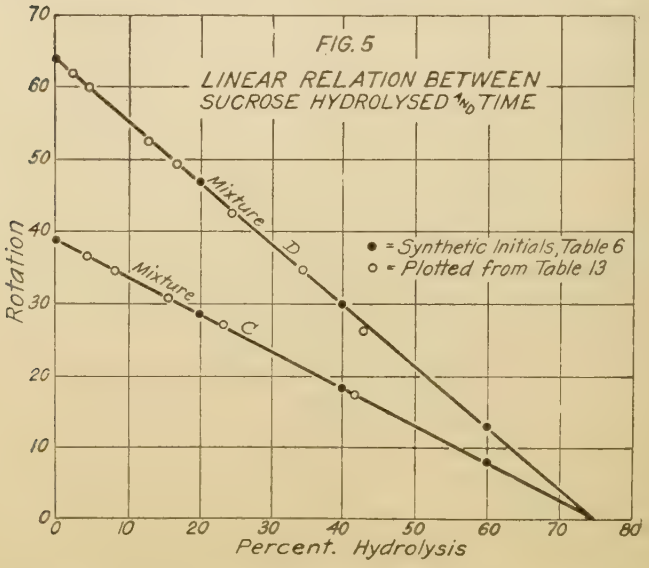
The results obtained showed that the use of the polari-

scope is permissible in following the hydrolysis of concentrated sucrose solutions.

TABLE VI.

Per Cent Hydrolysis	00%	20%	40%	60%
Rotation*, Mixture C	38.82	28.48	18.26	8.13
Rotation*, Mixture D	63.80	46.77	29.82	12.99

*For "Absolute Rotations," see page 25.



THE USE OF A HIGH BUFFER CONCENTRATION

It will be noticed that a rather high concentration of buffer solution was used in these experiments. This was done in order to maintain the hydrogen ion concentration as constant as possible over the entire range of sucrose concentrations.

Using the same M/20 citrate buffer, the PH's for the various mixtures were found to be:—

a	b	A	AA	B	BB	C	CC	D	E
4.99	5.00	5.00	5.02	5.04	5.05	5.06	5.07	5.08	5.10

That this change in the PH due to a change in sucrose concentration does not affect the direct comparability of the velocities (i.e., that we may consider the PH of all the hydrolysis mixtures as 5.00) was subsequently determined in a series of experiments on the effect of PH on velocity (PART III). In the neighborhood of PH 5.00 the greatest variation in velocity, due to a change in PH, was found to be in the case of the dilute solutions, but it became smaller as the sucrose concentration increased. At the higher sucrose concentrations the velocity of hydrolysis at PH 5.00 was not affected within experimental error by a change in PH of 0.1, as can be seen from figure 11.

The necessity of employing a higher buffer concentration than is customary is shown by a comparison of the PH's obtained for the two sucrose concentrations (10 per cent and 35 per cent) using M/20 buffer solution with those obtained when M/100 buffer solution was used. These are given in table 7.

The use of such a high buffer concentration, however, occasioned a concomitant effect,—namely, a retardation of the velocity of hydrolysis due to the presence of appreciable amounts of salt (secondary sodium citrate) in solution. The extent of this retardation is indicated in table 7, wherein are tabulated the results of initial velocity determinations for two sucrose concentrations—namely, 10 per cent and 35 per cent—using invertase B with M/100 buffer and the initial velocities (as read off the PH velocity curves, figure 11) for the corresponding PH's when invertase B was used with M/20 buffer. The initial velocities obtained with the M/100 buffer are also shown graphically by two points plotted in figure 11. Taking the values given in table 7 it may be seen that the per cent retardation for

the 10 per cent sucrose solution using M/20 buffer, as compared to when M/100 buffer was used, is 4.5 per cent and for the 35 per cent solution is 4.0 per cent. This effect is comparable to the retardation effects described by Washburn[8] in using potassium chloride.

TABLE VII.
EFFECT OF BUFFER CONCENTRATION ON PH AND ON VELOCITY

Mixture	Sucrose Concent. %	M/20 Buffer Veloc. °/hr.	M/20 Buffer PH	M/100 Buffer Veloc. °/hr.	M/100 Buffer PH	Velocity using M/20 Buffer at corresponding pH	Change Velocity due to Buffer Concent.
10	10	5.78	5.005	5.88	5.29	5.62	.26
C	35	3.28	5.06	3.26	5.47	3.12	.14

THE VOLUME OBTAINED WHEN THE SODIUM CARBONATE SOLUTION IS ADDED TO THE HYDROLIZING MIXTURE

It was thought that an appreciable error might be introduced due to the volume-concentration phenomena manifested by aqueous sucrose solutions. That is, it is well known that if a determined volume of sucrose is added to a definite volume of water, the total volume of the resulting solution will be less than the sum of the two component volumes. We make the assumption below that the comparability of the volume-contractions for the 25 cc hydrolyzing samples and the 35 cc diluted (with sodium carbonate solution) hydrolysis samples will not be affected by the small quantity of salt and invertase present in solution.

On plotting the values, as given by Browne,[14] for this contraction against the sucrose concentration we obtain a curve (figure 6) with a maximum at a sucrose concentration of about 60 per cent. With this curve as a basis we can then read off the volume contraction values per liter for the different hydrolysis mixtures and, by dividing these

values by 40, obtain the volume contraction values for 25 cc (the volume of the hydrolysis samples) of sucrose solution.

In order to obtain the sucrose concentrations of the diluted (by 10 cc of sodium carbonate solution) hydrolysis samples we multiply the respective concentrations by 5/7. This gives us an approximation close enough to obtain contraction values per liter for the sucrose concentrations in the diluted hydrolysis samples. But in this case we are dealing with 35 cc of solution, instead of 25 cc as above, and are interested in the volume contraction values for 35 cc of solution. This is obtained by dividing the value for contraction per liter by 28.6.

A comparion of these two sets of values, given in table 8, shows that the amount of volume contraction for 25 cc of hydrolysis mixture is practically the same as for 35 cc of the diluted hydrolysis sample for all concentrations except those of mixtures D and E, the differences here being 0.03 cc and 0.08 cc respectively. It merely shows that, up to sucrose concentrations of about 50 per cent, two parts of water may be added to five parts of sucrose solution without incurring any appreciable volume contraction in the resulting solution as compared to the sum of the volumes of the initial sucrose solution and the water.

As the difference in volume contraction in the case of the most concentrated solution, E, is on the border of the limit of error (.10 35—0.28%) no corrections need be applied to any of the mixtures and the true changes in rotation for all mixtures can be directly obtained by multiplying the observed changes in rotation, as given by the polariscope, by the factor 7/5.

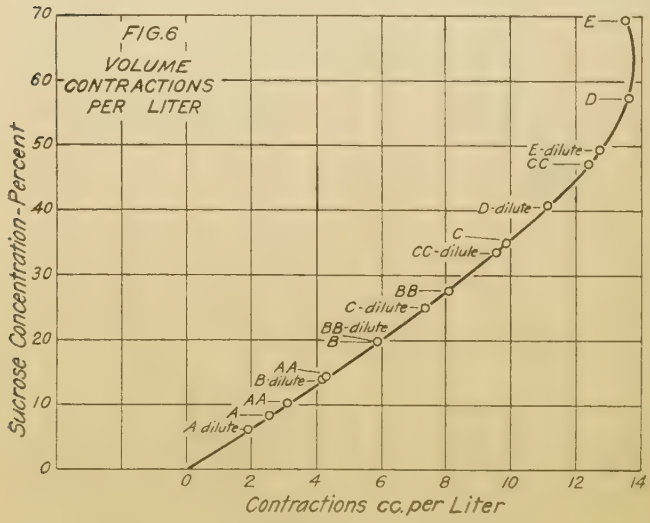
All the rotations given in this paper are stated as they were observed on the polariscope and hence should be multiplied by this factor if it is desired to obtain the absolute rotations. In compiling the tables for this investigation

this was not done as it was the relative, rather than the absolute, manner in which the velocities changed that was of interest.

TABLE VIII.

VOLUME CONTRACTIONS

Mixture	Hydrolysis Solution		25 cc Hydrolysis Solution Diluted with 10 cc of Sodium Carbonate Solution	
	Sucrose Concentration %	Volume Contraction per 25 cc cc	Sucrose Concentration %	Volume Contraction per 35 cc cc
A	8.435	.06	6.025	.06
AA	14.218	.11	10.155	.11
B	19.575	.15	13.982	.15
BB	27.680	.20	19.771	.20
C	34.917	.25	24.941	.26
CC	47.144	.31	33.674	.33
D	57.198	.34	40.856	.39
E	69.140	.34	49.385	.44



THE EFFECT OF VISCOSITY ON THE VELOCITY OF HYDROLYSIS AND A COMPARISON OF THE RESULTS OF THIS AND PREVIOUS INVESTIGATIONS

(a)

The relation of viscosity to the speed of hydrolysis of sucrose by invertase has been studied by Herzog [15] and by Colin and Chaudun,[5] as previously mentioned. As we could not see the significance of the results of Herzog and as our velocity determinations are in disagreement with those of Colin and Chaudun, the effect of viscosity was here studied again by two series of experiments, the hydrolysis mixtures in the second series being exactly the same as those in the first series except for the addition of a small amount of gelatine, which substance was used as a viscosifying agent taking no part in the reaction proper. This property of gelatine was previously indicated by the work of Nelson and Kerr.[16]

The relative viscosities of all mixtures were determined by means of an Ostwald viscosimeter and in table 9 are expressed in seconds of time. The observed initial velocities are expressed in degrees change in rotation per hour. It may be seen that the respective velocities for the different sucrose concentrations were not materially affected by an increase in viscosity approximating 20 per cent in each case.

TABLE IX.

	No Gelatine Added		1.1193 grams of Gelatine added per batch	
Mixture*	Velocity °/hr.	Viscosity seconds	Velocity °/hr.	Viscosity seconds
b	2.10	73.3	2.08	88.3
B	1.45	107.7	1.44	128.9
C	1.03	189.8	.99	223.8

*The compositions of these mixtures correspond to those of the same letter-symbol in Table I.

According to the claims of Colin and Chaudun, a change

of fluidity (fluidity equals the reciprocal of the viscosity) in the hydrolysis mixture should produce a corresponding change in velocity and the ratio of the change in velocity to the change in fluidity should always give a constant. Using the above results no such constant was found to exist. It might also be pointed out that, whereas the Δfluidity—Δsucrose concentration relationship of Colin and Chaudun (figure 8) practically agrees with a Δfluidity—Δconcentration relationship calculated from the data of Powell,[17] the velocity curve of these investigators is at decided variance from the findings in this investigation (figure 7).

(b)

In table 10 are given the observed initial velocities of hydrolysis for different sucrose concentrations as determined by Euler and Myrbaeck, by Colin and Chaudun, and in the present investigation.

It will be seen that the velocities have been measured according to different units in these three investigations. All of these units may, however, be transformed from one to the other by the use of a constant factor (according to the definition of velocity, page 6) and thus the relative change in velocity with sucrose concentration, as determined by these several investigations, may be compared directly by the use of the velocity units as given. In order to facilitate a graphical comparison (figure 7) of the results obtained in this laboratory with those obtained by Colin and Chaudun and with those by Euler and Myrbaeck, arbitrary factors have been applied to each series of velocities in order to bring the velocity-concentration curves closer together on the graph. These arbitrary factors are as follows:

For velocities obtained with invertase A1	X50
For velocities obtained with invertase B	X20
For velocities obtained by Euler and Myrbaeck	X50
For velocities obtained by Colin and Chaudun	X 1

The velocity scale on the graph is therefore an arbitrary one.

FIG. 7

COMPARATIVE OBSERVED INITIAL VELOCITIES

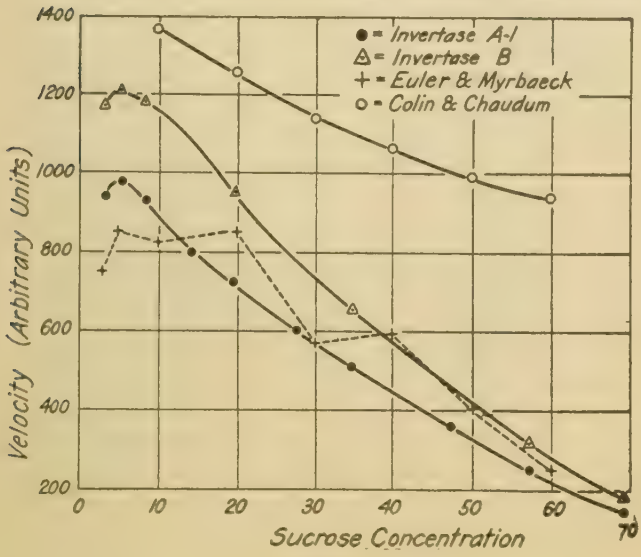


TABLE X.

This Investigation					
Sucrose Concentration %	Observed Velocity °/hr.		Sucrose Concent. %	Euler and Myrbaeck Observed Velocity °/min.	Colin and Chaudun Observed Velocity cgms/30 min.
	Invertase A-1	Invertase B			
3.48	1.88	5.88	3	1.50	—
5.37	1.95	6.04	5	1.70	—
8.44	1.86	5.90	10	1.65	137.0
14.22	1.60	—	20	1.70	125.5
19.58	1.45	4.76	30	1.14	114.0
27.68	1.20	—	40	1.19	106.5
34.92	1.03	3.28	50	.81	99.0
47.14	.72	—	60	.50	94.0
57.20	.50	1.60			
69.14	.29	.91			

(c)

In table 11 below the velocity determinations of this investigation are given together with those of Colin and Chaudun. The changes in velocity with sucrose concentration are also shown. These results are plotted in figure 8. For the purposes of comparison of the two curves representing the change in velocity with the change in sucrose concentration, the changes in velocity as given by Colin and Chaudun have been divided by 3. The ΔV scale in figure 8 is therefore an arbitrary one.

As has been noted above in section a, the data of Powell,[11] for the viscosity of sucrose solutions, has been used for the purposes of this investigation in computing the fluidity and change in fluidity for different sucrose concentrations. In table 12 below are given the fluidity and change-in-fluidity values for different sucrose concentrations as given by Colin and Chaudun and as computed from the data of Powell. The relationship between sucrose concentration and change in fluidity is also plotted in figure 8. The changes in fluidity from Powell's data are divided by 4 and hence the ΔF scale is also an arbitrary one.

The apparency of the fluidity-velocity relationship is manifest by a study of the curves in figure 8, showing the change in velocity and the change in fluidity plotted against the sucrose concentration. These curves run approximately parallel between sucrose concentrations of 20 per cent and 40 per cent. Their divergence, however, becomes quite noticeable at the higher sucrose concentrations. The change-in-fluidity curve of Colin and Chaudun has practically the same curvature as that constructed from the data of Powell. The two are not quite super-imposable, however. On the other hand, the curvature of the change-in-velocity of Colin and Chaudun is slightly greater than that based on duplicate determinations in this investigation.

TABLE XI.

VELOCITY-CONCENTRATION RELATIONSHIP

This Investigation			Colin and Chaudun		
Sucrose Concentration %	Velocity °/hr.	Δ V	Sucrose Concentration %	Velocity cgms/hr.	Δ V
10	1.82*	—	10	137	—
14.22	1.60	.22	20	125.5	11.5
19.58	1.45	.37	30	114	23
27.68	1.20	.62	40	106.5	30.5
34.92	1.03	.79	50	99	38
47.14	.72	1.10	60	94	43
57.20	.50	1.32			
69.14	.29	1.53			

*Note:—The velocities in this column are from Table II. and so represent Invertase A-1. The velocity for a ten per cent sucrose concentration at PH 5.00 was obtained by multiplying the velocity of a ten per cent solution with Invertase B at this PH (taken from Table XXIII.) by the conversion factor .315.

FIG. 8

RELATION OF CHANGE IN FLUIDITY AND CHANGE IN VELOCITY TO SUCROSE CONCENTRATION

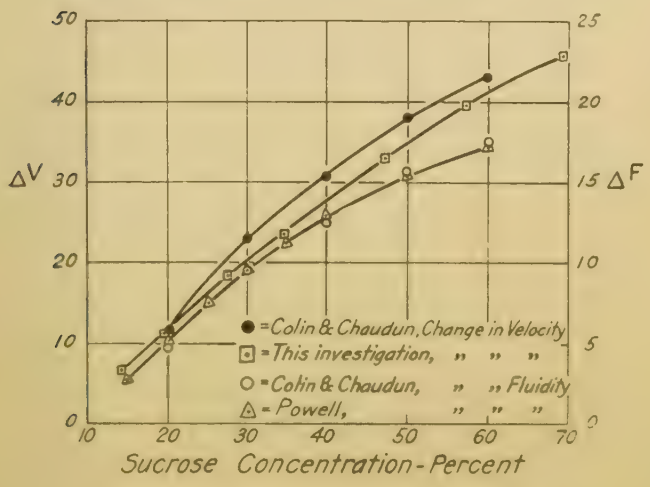


TABLE XII.

FLUIDITY-CONCENTRATION RELATIONSHIP

Fluidity equals the reciprocal of the viscosity.

Sucrose Concentration	Computed from the Data of Powell		Colin and Chaudun	
%	Fluidity	△ F	Fluidity	△ F
10	762	—	20	—
15	656	106		
20	557	205	15.35	4.65
30	382	380	10.51	9.49
40	246	516	7.46	12.54
50	147	615	4.46	15.54
60	79	683	2.56	17.44

THE COURSE OF THE HYDROLYSIS IN CONCENTRATED SUCROSE SOLUTIONS

Hitchcock[18] derived an empirical equation:

$$N = 1/t\ [\log 100/(100\text{-}p) + .002642p - .00000886p^2 - .0000001034p^3]$$

that describes the course of the hydrolysis of 10 per cent sucrose solutions by yeast invertase. The constancy of N in a particular hydrolysis is independent of the concentration of the invertase preparation used and its value is proportional to the concentration of the enzyme.

Post[19] showed that when this equation was applied to the hydrolysis of 10 per cent sucrose solutions to which invert sugar had been added, that the value of N was no longer constant but continually decreased with the progress of the reaction, while on the other hand 10 per cent sucrose solutions containing a-methyl glucoside gave increasing N's.

The course of the hydrolysis of high sucrose concentration has been studied from this point of view. As a matter of record it was found that, when Hitchcock's equation is applied to the hydrolysis of concentrated sucrose solutions, the value of N continually decreases, as was the case where a 10 per cent sucrose solution was hydrolyzed in the presence of added invert sugar. In other words additional suc-

rose appears to have an effect on the course of the hydrolysis similar to that of added invert sugar.

In table 13 below are given the complete hydrolysis figures for Mixtures B, C, and D, together with the computed N-values.

TABLE XIII.

MIXTURE B.

Sucrose Concentration = 19.58%

PH 5.04 Temperature 25° C. Invertase A-1†

Time	Rotation*	Change Rotation	Per Cent Hydrolyzed	N
00 mins.	(21.77)	—	—	—
30 mins.	21.04	.73	2.56	.03595
60 mins.	20.33	1.44	5.06	.03568
2 hrs.	18.86	3.29	10.22	.03639
4 hrs.	16.01	5.76	20.26	.03684
8 hrs.	10.87	10.90	38.28	.03649
12 hrs.	6.74	15.03	52.79	.03552
24 hrs.	−1.45	23.22	81.56	.03477
28 hrs.	−2.80	24.57	86.30	.03424
32 hrs.	−3.75	25.52	89.64	.03362
48 hrs.	−6.27	28.04	98.49	.03919
final	−6.70	28.47	100.00	—

*For "Absolute Rotations," see page 25.

†The original batch of Invertase A-1 was used here. See note at the head of Table II., Section B.

TABLE XIII.

MIXTURE C.

Sucrose Concentration = 34.92%

PH 5.06 Temperature 25° C. Invertase A-1†

Time	Rotation*	Change Rotation	Per Cent Hydrolyzed	N
00 mins.	(38.85)	—	—	—
30 mins.	38.32	.53	1.01	.01482
60 mins.	37.82	1.03	2.02	.01416
2 hrs.	36.77	2.08	4.07	.01431
4 hrs.	34.73	4.12	8.07	.01431

TABLE XIII. MIXTURE C.—*Continued.*

Sucrose Concentration = 34.92%

PH 5.06	Temperature 25° C.		Invertase A-1 †	
Time	Rotation*	Change Rotation	Per Cent Hydrolyzed	N
8 hrs.	30.84	8.01	15.69	.01412
12 hrs.	27.06	11.79	23.10	.01409
24 hrs.	17.49	21.36	41.85	.01345
28 hrs.	14.75	24.10	47.22	.01327
32 hrs.	12.06	26.79	52.49	.01320
36 hrs.	9.72	29.13	57.07	.01305
48 hrs.	3.98	34.87	68.32	.01261
60 hrs.	− .33	39.12	76.76	.01229
72 hrs.	−3.33	42.18	82.64	.01194
120 hrs.	−8.60	47.45	92.97	.01033
final	−12.19	51.04	100.00	—

*For "Absolute Rotations," see page 25.

†The original batch of Invertase A-1 was used here. See note at the head of Table II., Section B.

MIXTURE D.

Sucrose Concentration = 57.20%

PH 5.08	Temperature 25° C.		Invertase A-1†	
Time	Rotation*	Change Rotation	Per Cent Hydrolyzed	N
00 mins.	(63.79)	—	—	—
30 mins.	63.54	.25	.28	.00390
60 mins.	63.29	.50	.57	.00398
2 hrs.	62.79	1.00	1.14	.00398
4 hrs.	61.81	1.98	2.25	.00395
8 hrs.	59.81	3.98	4.54	.00400
12 hrs.	57.86	5.93	6.76	.00399
24 hrs.	52.41	11.38	12.98	.00387
28 hrs.	50.74	13.05	14.88	.00382
32 hrs.	49.01	14.78	16.86	.00380
36 hrs.	47.32	16.47	18.80	.00351
48 hrs.	42.47	21.32	24.33	.00372
60 hrs.	37.87	25.92	29.58	.00367
72 hrs.	33.65	30.14	34.39	.00360
96 hrs.	26.07	37.72	43.04	.00347
120 hrs.	24.87	38.92	44.41	.00288
final	−20.85	87.64	100.00	

*For "Absolute Rotations," see page 25.

†The original batch of Invertase A-1 was used here. See note at the head of Table II., Section B.

PART II.

THE EFFECT OF THE PRODUCTS OF HYDROLYSIS ON THE VELOCITY OF REACTION IN CONCENTRATED SUCROSE SOLUTIONS

The effect of the products of hydrolysis on the velocity of the sucrose-invertase reaction has been extensively studied for dilute sucrose concentration by Henri,[20] Barendrecht,[21] Michaelis and Menton,[2] Kuhn,[22] Euler and Josephson,[23] and Josephson,[24] as well as in this laboratory.[19, 25] European investigators have generally adopted the hypothesis of Michaelis that retardants like invert sugar produce their effect by combining with part of the enzyme and so leaving less to react with the sucrose. Bodansky,[25] on the other hand, studied the retardant action of the products of the invertase hydrolysis by considering an "Ideal Hydrolysis" of a 10 per cent sucrose solution, wherein the products of the reaction were removed as soon as they were formed, and comparing the velocities during the course of this "Ideal Hydrolysis" with those observed during the course of a normal hydrolysis, of a 10 per cent sucrose solution.

It was the aim of the present investigation to carry the study of the retardant action of the products of hydrolysis, glucose and fructose, into the region of concentrated sugar solutions.

This was done by four series of experiments in which the total sugar concentrations varied from about 3 per cent to about 70 per cent. In the first series 20 per cent of the total sugar was present as invert sugar and 80 per cent as sucrose. In the second series 40 per cent was invert sugar and 60 per cent sucrose, and in the third series 60 per cent was invert sugar and 40 per cent sucrose. In the fourth series of experiments a constant amount of invert sugar was in each case added to solutions containing different amounts of sucrose.

The amount of invertase added in all cases was the same. The amount of water added to each hydrolysis mixture was 200 cc less the amount of water represented in the invert sugar that was added,—that is, allowance was made for the fact that empirically invert sugar is one molecule of sucrose plus one molecule of water. The PH was maintained constant at 5.00 throughout all four series by the use of M/20 secondary sodium citrate buffer and the temperature kept at 25°C.

The volumes of the various hydrolysis mixtures in the first three series of experiments, wherein 20, 40, and 60 per cents of the sucrose used in the previous experiments was replaced by invert sugar, were taken to be the same as given in table 1 for the respective mixtures. A small error, due to the assumption that one molecule of invert sugar has a volume equal to the sum of the volumes of one molecule of sucrose plus one molecule of water, is undoubtedly here introduced. The magnitude of this error, as computed from the tables of Landolt-Boernstein[26] for the specific gravities of sucrose and invert sugar solutions, is less than 2 to 3 parts in 10,000, and therefore is negligible. The volumes and concentrations in the fourth series were computed from specific gravities that were determined in each case.

The same experimental procedure was used as has been given above with the addition that the buffer-sugar solutions were allowed to stand a sufficient length of time, as estimated from the data of Beegle,[9] to insure complete mutarotation of the glucose and fructose.

The data for the four series of experiments are as follows:—

TABLE XIV.

COMPOSITIONS OF MIXTURES WHEREIN 20, 40 AND 60 PER CENT OF THE SUCROSE IS REPLACED BY INVERT SUGAR*

Mixture	Total Sugar Concent %	% Sucrose Replaced by Invert Sugar	Sucrose Added gms	Glucose Added gms	Fructose Added gms	Buffer Used cc
		20	6.40	.8421	8.421	199.92
a	3.478	40	4.80	1.6842	1.6842	199.83
		60	3.20	2.5263	2.5263	199.75
		20	10.00	1.3158	1.3158	199.87
b	5.369	40	7.50	2.6316	2.6316	199.74
		60	5.00	3.9474	3.9474	199.61
		20	16.00	2.1053	2.1053	199.79
A	8.435	40	12.00	4.2106	4.2106	199.58
		20	40.00	5.2632	5.2632	199.47
B	19.575	40	30.00	10.5264	10.5264	198.94
		60	20.00	15.7896	15.7896	198.44
		20	80.00	10.5264	10.5264	198.95
C	34.917	40	60.00	21.0528	21.0528	197.90
		60	40.00	31.5792	31.5792	196.84
		20	160.00	21.0528	21.0528	197.90
D	57.198	40	120.00	42.1056	42.1056	195.79
		60	80.00	63.1584	63.1584	193.69
		20	220.00	28.9480	28.9480	197.11
E	69.140	40	165.00	57.8960	57.8960	194.22
		60	110.00	86.8440	86.8440	191.32

*25 cc of Invertase was used with all above mixtures.

TABLE XV. SECTION A.

INDIVIDUAL HYDROLYSES OF MIXTURES GIVEN IN TABLE XIV.

The invertase used for these experiments was from two different batches of Invertase A-1. In order to make the

results obtained comparable to those obtained for sucrose alone (as given in table 2) where the original batch of this invertase preparation was used, it has been necessary to employ the conversion factors .933 and .840 in a similar manner as described at the head of table 2B.

The numerical subscripts, following the symbol letter of the mixture used, signify the per cent of sucrose that has been replaced by invert sugar. Thus mixture A_{20} denotes the mixture given in the table above containing 16 gms. of sucrose, 2.1053 gms. of glucose and 2.1053 gms. of fructose.

The initial rotations are extrapolated values.

	Mixture a_{20}		Mixture a_{40}		Mixture a_{60}	
Time Minutes	Rotation*	Change	Rotation*	Change	Rotation*	Change
00	(2.85)	—	(1.86)	—	(0.85)	—
10	2.58	.27	1.63	.23	.68	.17
20	2.30	.55	1.40	.46	.52	.33
30	2.03	.82	1.17	.69	.35	.50
60	1.32	1.53	.59	1.27	−.05	.90

	Mixture b_{20}		Mixture b_{40}		Mixture b_{60}	
Time Minutes	Rotation*	Change	Rotation*	Change	Rotation*	Change
00	(4.37)	—	(2.84)	—	(1.33)	—
10	4.09	.28	2.61	.23	1.14	.19
20	3.80	.57	2.37	.47	.95	.38
30	3.53	.84	2.14	.70	.76	.57
60	2.69	1.68	1.43	1.41	.25	1.08

	Mixture A_{20}		Mixture A_{40}		Mixture A_{60}	
Time Minutes	Rotation*	Change	Rotation*	Change	Rotation*	Change
00	(6.90)	—	(4.49)	—		
10	6.61	.29	4.24	.25		
30	6.02	.88	3.74	.75		
60	5.16	1.74	3.02	1.47		
90	4.35	2.55	2.33	2.16		

TABLE XV. SECTION A.—*Continued.*

Time Minutes	Mixture B_{20} Rotation*	Change	Mixture B_{40} Rotation*	Change	Mixture B_{60} Rotation*	Change
00	(15.97)	—	(10.34)	—	(4.69)	—
30	15.33	.64	9.81	.53	4.26	.43
60	14.69	1.28	9.28	1.06	3.83	.86
90	14.06	1.91	8.74	1.60	3.41	1.28
120			8.22	2.12	2.98	1.71

Time Minutes	Mixture C_{20} Rotation*	Change	Mixture C_{40} Rotation*	Change	Mixture C_{80} Rotation*	Change
00	(28.48)	—	(18.26)	—	(8.13)	—
30	28.02	.46	17.88	.38	7.84	.29
60	27.57	.91	17.47	.79	7.55	.58
90	27.11	1.37	17.11	1.15	7.28	.85
120	26.64	1.84	16.73	1.53	6.99	1.14

Time Minutes	Mixture D_{20} Rotation*	Change	Mixture D_{40} Rotation*	Change	Rotation*	Change
00	(46.75)	—	(29.82)	—		
30	46.53	.22	29.65	.17		
60	46.30	.45	29.48	.34		
90	46.09	.66	29.31	.51		
150	45.64	1.10	28.98	.84		

Time Minutes	Mixture E_{20} Rotation*	Change	Mixture E_{40} Rotation*	Change	Mixture E_{80} Rotation*	Change
00	(55.86)	—	(35.33)	—	(15.08)	—
30	55.73	.13	35.22	.11	14.99	.09
60	55.60	.26	35.11	.22	14.91	.17
90	55.47	.39	35.00	.33	14.81	.27

*For "Absolute Rotation," see page 25.

TABLE XV. SECTION B.

INITIAL VELOCITIES FOR HYDROLYSES GIVEN IN SECTION A.

Batch No. 2 of invertase A-1 was used with Mixtures a, b, B, C, D, and E. To reduce velocities obtained with this batch to those that would have been obtained with the original batch, the factor X .933 is used.

Batch No. 3 of invertase A-1 was used with Mixture A, and the conversion factor here is .840.

Mixture	Observed Velocity °/hr.	Invertase Factor	Velocities Corresponding to use of original batch of Invertase A-1	Corrected Velocities °/hr.
a_{20}	1.64	.933	1.53	1.56
a_{40}	1.38	.933	1.29	1.32
a_{60}	1.00	.933	.93	.95
b_{20}	1.68	.933	1.57	1.62
b_{40}	1.41	.933	1.31	1.36
b_{60}	1.14	.933	1.06	1.10
A_{20}	1.75	.840	1.47	1.55
A_{40}	1.48	.840	1.24	1.31
B_{20}	1.28	.933	1.19	1.35
B_{40}	1.06	.933	.99	1.12
B_{60}	.86	.933	.80	.91
C_{20}	.92	.933	.86	1.09
C_{40}	.77	.933	.72	.92
C_{60}	.58	.933	.54	.69
D_{20}	.44	.933	.41	.64
D_{40}	.34	.933	.32	.50
E_{20}	.26	.933	.24	.42
E_{40}	.22	.933	.21	.37
E_{60}	.17	.933	.16	.28

FIG. 9

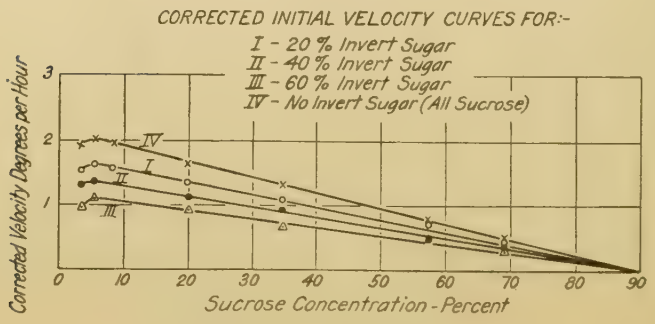


TABLE XVI

COMPOSITIONS AND DATA FOR HYDROLYSIS MIXTURES WHEREIN A CONSTANT AMOUNT OF INVERT SUGAR WAS ADDED IN EACH CASE.

All hydrolysis mixtures given in this table contained,—

Glucose ..... 22.5 gms.
Fructose ..... 22.5 gms.
M/20 Secondary Sodium Citrate Buffer 200 cc
Invertase B ..... 25 cc

in addition to the amounts of sucrose noted below.

Mixture	Sucrose Added gms.	Specific Gravity gms/cc	Total Volume of Solution cc	Total Sugar Conc. as Sucrose %	Water Concent. M/L*
A-1	20	1.09570	265.49	23.64	46.804
W-1	28	1.10480	270.55	26.15	46.063
X-1	40	1.11803	278.08	29.76	44.686
B-1	50	1.12918	284.19	32.64	43.725
Y-1	62.5	1.14168	292.03	36.04	42.552
BB-1	75	1.15303	299.99	39.25	41.422
C-1	100	1.17495	315.68	45.22	39.364
CC-1	150	1.21209	347.25	55.51	35.784
D-1	200	1.24217	379.10	64.03	32.779

*Note:—The abbreviation M/L signifies "Moles per Liter."

TABLE XVII. SECTION A.

INDIVIDUAL HYDROLYSES OF MIXTURES GIVEN IN TABLE XVI.

A-I			W-I		
Time Minutes	Rotation*	Change	Time Minutes	Rotation*	Change
00	(2.89)	—	00	(6.24)	—
10	2.52	.37	10	5.83	.41
30	1.79	1.10	20	5.43	.81
60	0.73	2.16	30	5.02	1.22
90	−.24	3.13	60	3.80	2.44

TABLE XVII. SECTION A.—*Continued.*

Time Minutes	X-I Rotation*	Change
00	(10.66)	—
10	10.22	.44
20	9.78	.88
30	9.35	1.31
60	8.13	2.53

Time Minutes	B-I Rotation*	Change
00	(14.35)	—
10	13.93	.42
20	13.50	.85
30	13.08	1.27
60	11.88	2.47

Time Minutes	Y-I Rotation*	Change
00	(18.60)	—
10	18.19	.41
20	17.78	.82
30	17.37	1.23
60	16.20	2.40

Time Minutes	BB-I Rotation*	Change
00	(22.64)	—
10	22.25	.39
20	21.87	.77
30	21.47	1.17
60	20.35	2.29

Time Minutes	C-I Rotation*	Change
00	(30.40)	—
10	30.06	.34
20	29.71	.69
30	29.37	1.03
60	28.41	1.99

Time Minutes	CC-I Rotation*	Change
00	(43.53)	—
10	43.28	.25
20	43.02	.51
30	42.78	.75
60	42.02	1.51

Time Minutes	D-I Rotation*	Change
00	(54.32)	—
10	54.15	.17
20	53.97	.35
30	53.80	.52
60	53.29	1.03

*For "Absolute Rotations," see page 25.

TABLE XVII. SECTION B.

INITIAL VELOCITIES FOR HYDROLYSES GIVEN IN SECTION A.

Mixture	Observed Velocity °/hr.	Corrected Velocity °/hr.
A-1	2.20	2.60
W-1	2.44	2.93
X-1	2.62	3.24
B-1	2.54	3.20
Y-1	2.45	3.18
BB-1	2.34	3.12
C-1	2.06	2.89
CC-1	1.51	2.33
D-1	1.03	1.74

DISCUSSION

A graphical representation of the results obtained is shown in figures 9 and 10. By comparing curves I, II, and III (the initial velocity curves obtained when respectively 20 per cent, 40 per cent and 60 per cent of the sucrose is replaced by invert sugar), in figure 9 with curve IV (the initial velocity curve for sucrose alone), it may be seen that the invert sugar appears to reduce the velocities obtained with different concentrations of sucrose alone by the same fractional amount when the fraction of sucrose replaced by invert sugar is constant,—thus giving us the same velocities that would be obtained by merely using less invertase. In other words it would appear that the invert sugar renders part of the invertase inactive and the fraction of the invertase that is so rendered is dependent on the ratio of the invert sugar to the sucrose in solution.

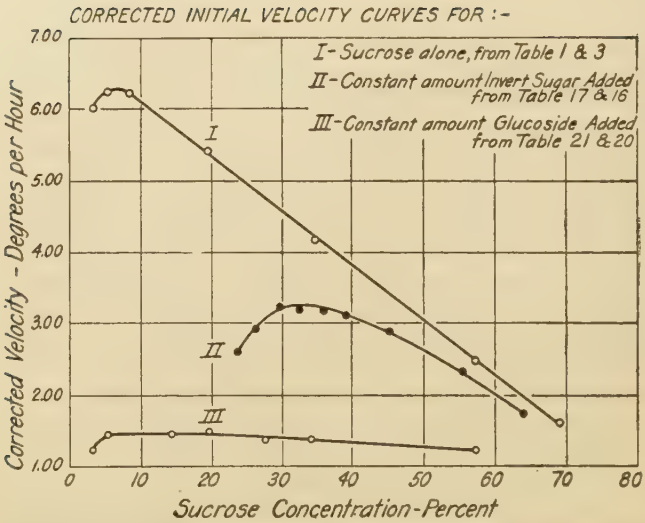
This relationship seems also to be borne out by a comparison of the results (plotted in curve II, figure 10) for a constant amount of invert sugar added to different amounts of sucrose in solution, with those obtained when sucrose alone was present. It may be seen that the invert sugar has less and less effect on the velocity of hydrolysis as its ratio to the sucrose becomes less and less.

If one were to adopt the hypothesis of Michaelis that invert sugar retards by combining with part of the invertase, then the present data would appear to support this hypothesis.

THE RETARDANT EFFECT OF A-METHYL GLUCOSIDE ON THE VELOCITY OF HYDROLYSIS BY INVERTASE OF CONCENTRATED SUCROSE SOLUTIONS

It has been pointed out by Nelson and Freeman[27] and Post[19] that the effect of a-methyl glucoside on the activity of invertase, as measured by the velocity of hydrolysis, is of a different nature than that of invert sugar. That is, both of these substances retard the velocity of this hydrolysis, but in a manner which is so different that it leads us to

FIG. 10



believe that different parts of the enzyme are affected by the glucoside than by the invert sugar.

Accordingly it was felt desirable to see if this same difference in the manner of retardation manifested itself in the case of more concentrated sucrose solutions. For this purpose a series of hydrolyses were run wherein each mixture contained a constant amount of glucoside equal to 1 per cent of the weight of the water present.

The results are recorded below in tables 18-21.

TABLE XVIII.

COMPOSITION OF THE GLUCOSIDE MIXTURES

The compositions of the mixtures used in these experiments are the same as those denoted by a corresponding letter-symbol in table 1, with the exception that 2.2387 grams of a-methyl glucoside was added in each case. This amount of glucoside represents 1 per cent of the weight of water present in each hydrolysis mixture. Such a mixture is signified as containing 1 per cent glucoside.

TABLE XIX

SPECIFIC GRAVITIES OF THE GLUCOSIDE MIXTURES

The specific gravities used in these experiments were not directly determined but taken to be the mean of the specific gravities (determined in a previous series of experiments) for 2 per cent glucoside mixtures and those where no glucoside was present (as given in table 1). It may be seen that the total difference between the corresponding specific gravities in these two sets of values is less than one part in a hundred even for the most dilute sugar solutions. In the case of mixture BB the difference is only one part in ten thousand. It was therefore felt justifiable to use the extrapolated values.

Mixture	Specific Gravity, no glucoside present	Specific Gravity, 2% glucoside present	Extrapolated Specific Gravity for 1% glucoside mixtures
a	1.01670	1.02226	1.01948
b	1.02623	1.02931	1.02777
A	1.03709	1.04057	1.03882
AA	1.05988		1.06138
B	1.08021		1.08034
BB	1.11054	1.11067	1.11060
C	1.13793		1.13797
D	1.21806		1.21809

TABLE XX

DATA FOR GLUCOSIDE MIXTURES

Mixture	Specific Gravity, gms/cc	Total Volume cc	Sucrose Concentration %	Water Concentration M/L*
a	1.01948	231.63	3.45	53.646
b	1.02777	234.14	5.34	53.071
AA	1.06138	247.92	14.12	50.269
B	1.08034	257.48	19.42	48.260
BB	1.11060	272.95	27.48	45.525
C	1.13797	288.36	34.68	43.092
D	1.21809	351.48	56.90	35.353

*Note:—The abbreviations M/L Signifies "Moles per Liter."

TABLE XXI. SECTION A.

INDIVIDUAL HYDROLYSES OF GLUCOSIDE MIXTURES

Time Minutes	a-G Rotation*	Change
00	(6.41)	—
10	6.18	.23
20	5.96	.45
30	5.73	.68
60	5.03	1.38

Time Minutes	b-G Rotation*	Change
00	(8.47)	—
10	8.24	.23
20	8.02	.45
30	7.78	.69
60	7.09	1.38

Time Minutes	AA-G Rotation*	Change
00	(18.07)	—
10	17.85	.22
30	17.41	.66
60	16.76	1.31
90	16.11	1.96

Time Minutes	B-G Rotation*	Chagne
00	(23.94)	—
10	23.69	.25
30	23.30	.64
60	22.65	1.29
90	22.01	1.93

Time Minutes	BB-G Rotation*	Change
00	(32.72)	—
10	32.54	.18
30	32.16	.56
60	31.59	1.13
90	31.03	1.69

Time Minutes	C-G Rotation*	Change
00	(40.52)	—
10	40.35	.17
30	39.99	.53
60	39.45	1.07
90	38.93	1.59

Time Minutes	D-G Rotation*	Change
00	(64.89)	—
30	64.50	.39
60	64.11	.78
90	63.74	1.15
120	63.34	1.55

*For "Absolute Rotations," see page 25.

TABLE XXI. SECTION B.

INITIAL VELOCITIES FOR HYDROLYSES GIVEN IN SECTION A.

Mixture	Observed Velocity °/hr.	Corrected Velocity °/hr.
a-G	1.37	1.41
b-G	1.38	1.44
AA-G	1.31	1.44
B-G	1.29	1.48
BB-G	1.13	1.37
C-G	1.07	1.37
D-G	.78	.22

Curve III in figure 10 shows the initial corrected velocities obtained in these experiments, plotted against the corresponding sucrose concentrations. Between sucrose concentrations of 5 per cent and 35 per cent the presence of the a-methyl glucoside seems to annul the previously found retardant effects that accompany increasing sucrose concentrations. The results obtained are in agreement with the tentative hypothesis proposed by Post.[19]

PART III.

THE EFFECT OF SUCROSE CONCENTRATION ON THE PH--VELOCITY RELATIONSHIP

Michaelis and Davidson[28] showed that the effect of PH on the activity of Invertase, as measured by the velocity of hydrolysis of sucrose, was similar in nature to the effect of PH on the undissociated fraction of an ampholyte in aqueous solution. From this they developed their hypothesis that the velocity of hydrolysis is dependent on the amount of undissociated invertase-acid in the hydrolyzing mixture.

In a subsequent investigation in which they used sucrose concentration up to 26 per cent Michaelis and Menton[2] showed that when the logarithm of the sucrose concentration was plotted against the velocity of hydrolysis, they obtained a curve similar in nature to an ordinary "dissociationrest" curve. The term "dissociationrest" as given by Michaelis[29] signifies the undissociated portion of a dissociating compound existing in solution at equilibrium. A "dissociationrest" curve is one where the amount of the undissociated portion of the dissociating compound, existing in solution at equilibrium, is plotted against the logarithm of the concentration of that component which is being varied,—the total concentration (free and combined) of the other component remaining constant. Hence these investigators formulated the idea that the velocity of hydrolysis was likewise dependent on the amount of sucrose-invertase compound in solution.

The results of these two investigations are mathematically discussed by Michaelis in a later paper.[30]

In consideration of the previous studies of Michaelis and his co-workers, Michaelis and Rothstein[31] pointed out theoretically that the PH-velocity curve for invertase hydrolyses should be shifted to the right with increasing sucrose concentrations, but in working with sucrose concentrations up to 15 per cent they were unable to detect any such shift experimentally. To explain this discrepancy between their experimental results and their theoretical calculations, they proposed a new hypothesis which said that sucrose and invertase form a compound which is itself an acid, and it is the undissociated portion of this sucrose-invertase acid that breaks up according to a monomolecular reaction into invertase, fructose, and glucose.

Euler, Josephson and Myrbaeck[32] combined the ideas of Michaelis and Davidson and of Michaelis and Rothstein and suggested that the difficulty, due to the difference between theory and experiment, is solved if the acid dissociation of "free invertase" as well as that of the sucrose-invertase compound is taken into account. Their results showed that the relative velocities (velocity at PH 5 being taken as one), taken from an experimentally determined PH-velocity curve, agree with the relative amounts of the undissociated invertase-sucrose compound in solution as computed from their theoretical equation. The most concentrated sucrose solution in the above consideration is 6.8 per cent. Kuhn[33] comments that this theory should be considered with some reservation as it only takes into account part of the significance of the PH-velocity curve, and he further comments[34] that any explanation of the shape of the PH-velocity curves must include the significance of "the electrochemical properties of the enzyme and substrate, the rate of formation of the intermediate reaction products, the manner of diffusion of the cleavage products of the enzyme particles, and perhaps also the condition of hydration of the sugar compared

to that of the enzyme,—all of which change with the acidity."

METHOD AND RESULTS OF PRESENT INVESTIGATION

The effect of sucrose concentration on the PH-velocity relationship has been here studied over much wider variations in sucrose concentration than has been done heretofore,—namely sucrose concentrations of 10 per cent, 35 per cent, and 57 per cent,—and PH-velocity curves were obtained for each concentration between PH 3.3 and PH 7.5.

Our results show that, in relation to the 10 per cent curve, the curve for 35 per cent sucrose concentration was shifted 0.20 PH to the right and the curve for 57 per cent sucrose concentration about 0.35 PH to the right. That this small shift in the optimum could not be detected over the relatively small ranges of sucrose concentration heretofore investigated is not to be wondered at.

The manner of determining this shift in the PH-velocity curves is of some interest as the PH-velocity (figure 11) curves at high sucrose concentrations have such flat maximums that the usual method of estimating the optimum PH by sight is of little value. However we can with good accuracy estimate the maximum velocity for each concentration. Taking the maximum velocity of the 10 per cent curve as unity, proportionality factors are obtained for the maximum velocities shown by the 35 per cent and 57 per cent curves, and by means of these factors the 10 per cent curve is reduced to magnitudes that correspond respectively to the magnitudes of the velocities for the more concentrated mixtures. These reduced curves are shown in figure 11 by dotted lines. It can be seen from the graph that the PH shift from the reduced 10 per cent curves can be readily estimated at the steeper right hand portion of the curve and that it is very approximately 0.20 of a PH for the 35 per cent sucrose concentration and 0.35 of a PH for the 57 per cent sucrose concentration.

Thus the present investigation, which is over a much wider range of sucrose concentrations than has heretofore been investigated, shows that there is a shift of the PH-velocity curve with increasing sucrose concentration, that this shift is to the right, and that its magnitude is too small to be perceptible unless large differences of sucrose concentration are employed.

These results do not agree with the proposals of Euler and his co-workers, who said that the total amount of invertase bound to the cane sugar is independent of the hydrogen-ion concentration, and that the velocity of hydrolysis depended only on the undissociated fraction of this invertase-sucrose compound, in solution. Their equation shows that the amount of this compound should increase with increasing sucrose concentration. Thus, assuming that the velocity of hydrolysis is directly proportional to the amount of undissociated invertase-sucrose compound in solution, then the velocity should increase with increasing sucrose concentrations. As has been previously mentioned on page 5, Euler, Josephson and Myrbaeck tried to show that the velocity actually remained constant between sucrose concentrations of 5 per cent and 20 per cent. It has been shown by this investigation, however, that no such constancy of velocity occurs over this range of sucrose concentrations.

Again, Euler, Josephson and Myrbaeck, in computing the relative velocities take the velocity obtained at PH 5.00 as unity, for all hydrogen-ion concentrations. Inasmuch as these investigators only worked over a small range of comparatively dilute sucrose solution, this assumption would not produce any detectable error, for there would be no experimentally detectable shift in the PH-velocity curve over this small range. This assumption, however, would not be valid over such an extended range of sucrose concentration as has been here investigated, since appreciable shifting of the PH-velocity curve occurs over wide variations of the sucrose concentration.

Our findings are more in alignment with the considerations of Kuhn, as given above on page 48, that the shape of PH-velocity curves cannot be explained by considering the respective concentration of hydrogen-ion and sucrose alone in the hydrolyzing mixture.

It is worthy to note, however, that the results of the present investigation confirm the shift in the PH-velocity curve with sucrose concentration, theoretically predicted by Michaelis in 1918-1920.

TABLE XXII.

COMPOSITION OF MIXTURES USED IN PH-VELOCITY DETERMINATIONS

A hydrolysis mixture representing a sucrose concentration of 10 per cent, designated as Mixture 10, was obtained by the addition of 24.059 gms. of sucrose to 200 cc of Buffer solution and 25 cc of Invertase solution. By extrapolation from table 1 between Mixtures A (sucrose=8.435 gms./100 cc solution) and AA (sucrose=14.218 gms./100 cc solution) the following data for Mixture 10 was computed:—Specific Gravity 1.04325 gms./cc, Volume 239.56 cc, Water Concentration 51.8871 M/L.

The data for Mixtures C and D have already been given in table 1.

TABLE XXIII. SECTION A.

INDIVIDUAL HYDROLYSES AT DIFFERENT HYDROGEN-ION CONCENTRATIONS FOR MIXTURES 10, C, AND D

A phosphate buffer was used for the hydrolyses above PH=7.00. The use of this buffer is treated more fully below on pages 59 and 61.

Invertase B was used throughout these experiments. Where there is an asterisk (*) it signifies that that hydrolysis was run with a stronger invertase,—designated as Invertase Bx3. The factor to reduce the velocities obtained with Invertase Bx3 to be comparable to those obtained with Invertase B was found to be X .320, for which the data are given in table 24.

The temperature in all cases was 25° C.

TABLE XXIII. SECTION A—*Continued.*

MIXTURE 10

PH 4.00

Time Minutes	Rotation*	Change
00	(11.07)	—
10	10.11	.96
20	9.14	1.93
30	8.17	2.90
40	7.23	3.84

PH 4.47

Time Minutes	Rotation*	Change
00	(11.07)	—
10	10.09	.98
20	9.11	1.96
30	8.13	2.94
40	7.18	3.89

PH 5.00

Time Minutes	Rotation*	Change
00	(11.07)	—
10	10.12	.95
20	9.14	1.93
30	8.18	2.89
40	7.23	3.84

PH 5.56

Time Minutes	Rotation*	Change
00	(11.07)	—
10	10.18	.89
20	9.28	1.79
30	8.39	2.68
40	7.52	3.55

PH 6.03

Time Minutes	Rotation*	Change
00	(11.06)	—
10	10.29	.77
20	9.52	1.54
30	8.74	2.32
40	7.99	3.07

PH 6.64

Time Minutes	Rotation*	Change
00	(11.08)	—
10	10.54	.54
20	10.02	1.06
30	9.49	1.59
40	8.96	2.12

*PH 7.54

(Phosphate Buffer)

Time Minutes	Rotation*	Change
00	(11.16)	—
05	10.87	.29
10	10.57	.59
15	10.29	.87
20	10.00	1.16

MIXTURE C

PH 3.23

Time Minutes	Rotation*	Change
00	(38.75)	—
10	38.30	.45
20	37.84	.91
30	37.41	1.34
60		

PH 4.00

Time Minutes	Rotation*	Change
00	(38.75)	—
10	38.20	.55
20	37.71	1.04
30	37.19	1.56
60	35.62	3.13

TABLE XXIII. SECTION A—*Continued.*

PH 4.50

Time Minutes	Rotation*	Change
00	(38.73)	—
10	38.17	.56
20	37.66	1.07
30	37.10	1.63
60	35.47	3.26

PH 5.06

Time Minutes	Rotation*	Change
00	(38.70)	—
10	38.17	.53
20	37.61	1.09
30	37.06	1.64
60	35.42	3.28

PH 5.57

Time Minutes	Rotation*	Change
00	(38.68)	—
10	38.17	.51
20	37.66	1.02
30	37.14	1.54
60	35.62	3.06

PH 6.05

Time Minutes	Rotation*	Change
00	(38.64)	—
10	38.17	.47
20	37.71	.93
30	37.25	1.39
60	35.86	2.78

PH 6.70

Time Minutes	Rotation*	Change
00	(38.63)	—
10	38.30	.33
20	37.97	.66
30	37.64	.99
60	36.66	1.97

*PH 7.40 (Phosphate Buffer)

Time Minutes	Rotation*	Change
00	(38.70)	—
10	38.18	.52
30	37.12	1.58
60	35.54	3.16
90	33.96	4.74

MIXTURE D

PH 3.28

Time Minutes	Rotation*	Change
00	(63.48)	—
10	63.24	.24
30	62.76	.72
60	62.04	1.44
90	61.33	2.15

PH 4.06

Time Minutes	Rotation*	Change
00	(63.39)	—
30	62.62	.77
60	61.86	1.53
120	60.33	3.06
180	58.80	4.59

PH 4.50

Time Minutes	Rotation*	Change
00	(63.49)	—
10	63.19	.30
30	62.68	.81
60	61.90	1.59
90	61.05	2.44

PH 5.10

Time Minutes	Rotation*	Change
00	(63.39)	—
30	62.56	.83
60	61.79	1.60
90	61.01	2.38
120	60.19	3.20

TABLE XXIII. SECTION A.—*Continued.*

PH 5.60			PH 6.01		
Time Minutes	Rotation*	Change	Time Minutes	Rotation*	Change
00	(63.40)	—	00	(63.26)	—
10	63.12	.28	30	62.56	.70
30	62.61	.79	60	61.85	1.41
60	61.80	1.60	120	60.43	2.83
90	61.01	2.39	180	59.02	4.24

*PH 6.67 (Phosphate Buffer)			*PH 7.30 (Phosphate Buffer)		
Time Minutes	Rotation*	Change	Time Minutes	Rotation*	Change
00	(63.40)	—	00	(63.48)	—
10	62.83	.57	10	63.11	.37
30	61.67	1.73	30	62.43	1.05
60	59.97	3.43	60	61.38	2.10
90	58.28	5.12	90	60.33	3.15

*For "Absolute Rotations," see page 25.

TABLE XXIII. SECTION B.

INDIVIDUAL VELOCITIES FOR HYDROLYSES GIVEN IN SECTION A.

Mixture	PH	Observed Velocity °/hr.	Invertase Factor	Velocity Corresponding to use of Invertase B
10	4.00	5.79	1.00	5.79
	4.47	5.88	1.00	5.88
	5.00	5.78	1.00	5.78
	5.56	5.36	1.00	5.36
	6.03	4.62	1.00	4.62
	6.64	3.18	1.00	3.18
	7.54	3.48	.326	1.13
C	3.23	2.70	1.00	2.70
	4.00	3.12	1.00	3.12
	4.50	3.26	1.00	3.26
	5.06	3.28	1.00	3.28
	5.57	3.07	1.00	3.07
	6.05	2.78	1.00	2.78
	6.70	1.98	1.00	1.98
	7.40	3.16	3.26	1.03
D	3.28	1.45	1.00	1.45
	4.06	1.53	1.00	1.53
	4.50	1.59	1.00	1.59
	5.10	1.60	1.00	1.60
	5.60	1.60	1.00	1.60

TABLE XXIII. SECTION B.—*Continued.*

Mixture	PH	Observed Velocity °/hr.	Invertase Factor	Velocity Corresponding to use of Invertase B
	6.01	1.41	1.00	1.41
	6.67	3.42	.326	1.11
	7.30	2.10	.326	.68

FIG. 11

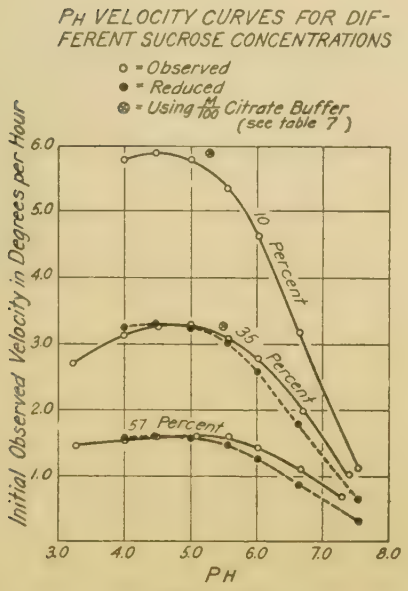


TABLE XXIV.

COMPARISON OF INVERTASE B WITH INVERTASE Bx3.

(Made with Mixture C, using phosphate buffer at PH 6.80

Invertase B			Invertase Bx3		
Time Minutes	Rotation*	Change	Time Minutes	Rotation*	Change
00	(38.67)	—	00	(38.73)	—
10	38.36	.31	10	37.74	.99
30	37.71	.96	20	36.75	1.98
60	36.74	1.93	30	35.77	2.96
120	34.81	3.86	40	34.77	3.96

*For "Absolute Rotations," see page 25.

TABLE XXIV.—*Continued.*

Initial Velocity Using Invertase B	1.93 °/hr
Initial Velocity Using Intervase Bx3	5.92 °/hr

Conversion Factor = 1.93/5.92 = .326

TABLE XXV.

REDUCED VELOCITIES OF THE 10 PER CENT PH-VELOCITY CURVE TO CORRESPOND WITH THE VELOCITY CURVES OF THE 35 PER CENT AND THE 57 PER CENT SUCROSE CONCENTRATIONS.

(See page 52.)

Maximum Velocity for 10% Mixture	5.88 °/hr.
Maximum Velocity for 35% Mixture	3.30 °/hr.
Maximum Velocity for 57% Mixture	1.60 °/hr.
Factor to obtain Reduced 35% Velocities	3.30/5.88 = .561
Factor to obtain Reduced 57% Velocities	1.60/5.88 = .272

PH	Velocity of 10% Mixture	Reduced Velocity for 35% Mixture	Reduced Velocity for 57% Mixture
4.00	5.79	3.25	1.57
4.47	5.88	3.30	1.60
5.00	5.78	3.24	1.57
5.56	5.36	3.01	1.46
6.03	4.62	2.59	1.26
6.64	3.18	1.78	.86
7.54	1.13	.63	.31

COMPARISON OF THE EFFECTS OF SUCROSE CONCENTRATION ON THE PH OF THE CITRATE BUFFER AND OF THE PHOSPHATE BUFFER

It is interesting to note that the effects of increasing sucrose concentration on the PH of the two types of buffers used were opposite in direction,—i.e., when the citrate buffer was used the PH of the sucrose solutions increased with increasing sucrose concentration while in the case of the phosphate buffer the PH decreased.

The effects due to sucrose concentration using a buffer solution of secondary sodium citrate of PH 5.00 have already been noted on page 20. The buffer action of this

citrate salt diminishes rapidly when the PH of the solution is lowered below 3.00 by the addition of hydrochloric acid or raised above 6.00 by the addition of sodium hydroxide solution.[35] In other words the range in which it will most effectively resist changes in the hydrogen ion concentration of the aqueous medium (changes due to such factors as the absorption of carbon dioxide from the air and fermentive effects on the acid-base equilibrium in the hydrolysis mixture proceeding simultaneously with the sucrose hydrolysis) lies between the limits of PH 3.00 and PH 6.00. Thus it might be expected the effect of sucrose concentration on the PH of the hydrolysis mixture would be considerably increased when the PH of the buffer solution was raised to the neighborhood of 7.0 by the addition of sodium hydroxide solution. This was found to be the case. Using the sodium citrate buffer to which sodium hydroxide solution was added to give a PH of about 7.00, the change in PH between zero per cent and 57 per cent sucrose concentration was .19, whereas using this same citrate buffer at PH 5.00 the variation was only .10 PH between these limits of sucrose concentration.

In an effort to minimize this variation in PH between different sucrose concentrations, a buffer composed of 3.8 parts of M/20 primary potassium phosphate solution and 6.2 parts of M/20 secondary sodium phosphate solution was used for hydrolysis represented at the extreme alkaline end of the PH-velocity curve. The data of Sorensen[35] shows this to be an effective buffer at this PH. In using this phosphate buffer, however, the amount of the PH shift was found to be greater than in the case where citrate buffer was used, the variation in PH between zero per cent and 57 per cent sucrose concentration being 0.60—0.70 with the phosphate buffer as compared to 0.19 PH when the citrate buffer was used in this same PH region.

A consideration of the principle underlying buffer-action, however, made it appear better judgment to use the phos-

phate buffer at these high PH's rather than the citrate buffer, as it was felt that the variation in PH was due to the effect of the large amounts of sucrose in solution on the aqueous concentration of the phosphate buffer salts, rather than the inability of the phosphate salt to buffer any particular mixture. Thus while an appreciable variation of the hydrogen-ion concentration might manifest itself as between different sucrose concentrations, the buffering action of the phosphate salts during a particular hydrolysis would probably still be better than that of citrate salt above PH 7.00. PH determinations on the individual hydrolysis mixtures, wherein the phosphate buffer was used, showed that no detectable change in the PH occurred during the time of hydrolysis.

The results are tabulated below:

TABLE XXVI.

Buffer No. 1—2000 cc M/20 secondary sodium citrate sol'n.
93 cc normal (approx) sodium hydroxide sol'n.
Buffer No. 2— 620 cc M/20 secondary sodium phosphate sol'n.
380 cc M/20 primary potassium phosphate sol'n.
Buffer No. 3—1000 cc Buffer No. 2.
240 cc N/20 (approx.) sodium hydroxide sol'n.

Sucrose Concentration %	00%	10%	35%	57%
PH Buffer No. 1	6.61	6.64	6.70	6.80
PH Buffer No. 2	7.07	—	6.80	6.67
PH Buffer No. 3	7.62	7.54	7.40	7.30

THE EFFECT OF THE VELOCITY OF HYDROLYSIS OF USING PHOSPHATE BUFFER INSTEAD OF CITRATE BUFFER

It was thought that the type of buffer salt used might have some effect on the velocity of hydrolysis, and accordingly such a comparison is here made.

It is evident from what has been said above (P. 57) regarding the comparative effectiveness of the citrate and the phosphate buffers used in this investigation, that the buffer action of the phosphate solution, against changes in in the hydrogen-ion concentration due to a change in suc-

rose concentration, is considerably less than the buffer action of the citrate solution in this respect even when the PH of the latter is carried beyond the limits of its most effective range of hydrogen-ion concentration. It was therefore felt that the buffer action of the phosphate salts in the case of individual hydrolyses would probably not be an effective regulator of the hydrogen-ion concentration at PH's other than in the immediate PH neighborhood of its most effective action, and it was for this reason that, in dealing with the comparative effects of the two types of buffer salt on the velocity of the invertase hydrolysis, the comparison was only made at that PH region where the phosphate buffer had its most effective action. In other words, as the phosphate solution here indicated itself to be such a relatively poor buffer in comparison to the citrate solution, it was felt that no purpose would be served by carrying this comparison of the relative effects of the two salts on the velocity of hydrolysis beyond the PH limit of the most effective buffer action of the phosphate salts.

The relative effect on the velocity of hydrolysis of these two buffer solutions (a description of these buffers is given on Page 22 and Page 57) was determined as follows:

Mixtures C and D were hydrolyzed with both citrate and phosphate buffer solutions that were so adjusted as to give approximately the same PH (6.70–6.80) for the two C mixtures and for the two D mixtures and the initial velocities determined. The rate of change of velocity with change in PH was ascertained from the slope of the PH-velocity curve (figure 11) at PH 6.7 and the velocities obtained with the phosphate buffer were corrected for the slight variations in PH between these mixtures and the mixtures in which the citrate buffer was used. As the differences in PH between the respective mixtures was small no appreciable error could be introduced in making the corrections above noted.

The results tabulated below showed that the velocities

obtained with the phosphate buffer were slightly higher than those obtained with the citrate buffer.

TABLE XXVII.

MIXTURE C

	PH	Velocity
Using Citrate Buffer	6.70	1.98
Using Phosphate Buffer	6.80	1.93

Slope of PH-velocity curve for Mixt. C at PH 6.70 = 1.19 degrees/PH
For $\triangle$ PH = 0.1, $\triangle$ velocity = .12 °/hr.
Velocity using phosphate buffer at PH 6.70 would be 2.05 °/hr.
Velocity using citrate buffer at PH 6.70 was 1.98 °/hr.

MIXTURE D

	PH	Velocity
Using Citrate Buffer	6.80	1.00
Using Phosphate Buffer	6.67	1.11

Slope of PH-velocity curve for Mixt. D at PH 6.70 = .60 degrees/PH
For $\triangle$ PH = .13, $\triangle$ Velocity = 0.08
Velocity using phosphate buffer at PH 6.80 would be 1.03 °/hr.
Velocity using citrate buffer at PH 6.80 was 1.00 °/hr.

MATERIALS

All the materials used in this investigation except the sugars have been described as they have been mentioned in the course of the paper. For a description of the preparation of the sucrose, glucose and fructose, see Bodansky,[35] and for the preparation of the a-methyl glucoside see Sottery.[36]

SUMMARY

The findings of this investigation may be summarized as follows:

1. It is evident from the results of this investigation that in working with solutions of high sucrose concentration, we must use a method of hydrolysis which eliminates certain gross experimental errors whose influences have been considered negligible at low sucrose concentrations. Such a method has been here described. The sources of the experimental errors that would be encountered in using the ordinary procedure in determining the velocities of hydrolysis have been carefully set forth, as well as the individual

effects of these errors on the velocity measurements. The method developed also allows us to account for the concentration of water, as well as that of invertase and of sucrose, in the hydrolysis mixture.

2. The results showed that undoubtedly the limiting factor of the velocity of hydrolysis at high sucrose concentrations is either the decreased water concentration or some retardant action of the sucrose itself when it occurs in great excess. Precisely which of these factors is the influential one could not be determined.

3. It has been shown that viscosity is not the principal factor for the dropping off of the hydrolysis velocities with increasing sucrose concentration.

4. It has been shown that, when Hitchcock's equation is applied to the hydrolysis of concentrated sucrose solutions, decreasing N's are obtained.

5. In the case of the retardant action of the products of hydrolysis the evidence here given would seem to agree with the hypothesis of Michaelis that retardation by invert sugar is due to compound formation between the enzyme and the invert sugar. The evidence also suggests the idea that the presence of mutarotated invert sugar in the hydrolysis mixture produces an effect on the initial velocity similar to that produced when less invertase is used. This effect on the initial velocity is not to be confused with the effect of added invert sugar on the course of the hydrolysis, for it has been previously pointed out that the effect on the course of hydrolysis of less invertase and of added invert sugar are of different natures.

6. The studies with a-methyl glucoside were an extension of the work of previous investigators, carrying the observed effects into the region of higher sucrose concentrations. The presence of a-methyl glucoside in the hydrolyzing mixture seems to annul the retardant effects experienced with increasing sucrose concentrations.

7. It has been shown that the concentration of sucrose bears a definite significance to the PH-velocity relationship.

REFERENCES

[1] Browne, Jour. Chem. Soc. **81,** 375 (1902).

[2] Michaelis and Menton, Biochem. Zeit. **49,** 333 (1913).

[3] Euler and Myrbaeck, Zeit. f Physiol. Chem. **124,** 159 (1922)

[4] Nelson and Vosburgh, Jour. Amer. Chem. Soc. **39,** 790 (1917)

[5] Colin and Chaudun, Jour. de Chim. Phys. **20,** 471 (1923)

[6] For a description of the procedure generally used in this laboratory see Nelson and Bloomfield, Jour. Amer. Chem. Soc. **46,** 1025 (1924)

[7] Nelson and Hollander, Jour. Biol. Chem. **58,** 291 (1923)

[8] Washburn, Dissertation, Columbia University, 1925.

[9] Nelson and Born, Jour. Amer. Chem. Soc. **36,** 393 (1914)

[10] D. J. and J. J. Beaver, Ind. and Chem. Eng. **15,** 359 (1923)

[11] Beegle, Dissertation, Columbia University, 1918.

[12] Rosanoff, Jour. Amer. Chem. Soc. **33,** 1911 (1911)

[13] Vosburgh, Jour. Amer. Chem. Soc. **43,** 219 (1921)

[14] Browne, Handbook of Sugar Analysis (1912), Table VIII, P. 34.

[15] Herzog, Zeit. f Phys. Chem. **43,** 222 (1904)

[16] Nelson and Kerr, Jour. Biol. Chem. **59,** 495 (1924)

[17] Powell, Jour. Chem. Soc. **105,** 1 (1914)

[18] Hitchcock, Dissertation, Columbia University, 1922.

[19] Post, Dissertation, Columbia University, 1925.

[20] Henri, Lois générales de l'action des diastases, Paris, 1903.

[21] Barendrecht, Zeit. Phys. Chem. **49,** 456 (1904), **54,** 367 (1905)

[22] Kuhn, Zeit. Physiol. Chem. **127,** 234 (1923), **129,** 57 (1923), **135,** 1 (1923-24)

[23] Euler and Josephson, Zeit. f Physiol. Chem. **132,** 301 (1923-24)

[24] Josephson, Zeit. Physiol. Chem. **134,** 50 (1923-24)

[25] Bodansky, Dissertation, Columbia University, 1924.

[26] Landolt-Boernstein, Tabellen, 5th Auflage, Band 1, Pp. 465 and 468.

[27] Nelson and Freeman, Jour. Biol. Chem. **63,** 365 (1925)

[28] Michaelis and Davidson, Biochem. Zeit. **35,** 386 (1911)

[29] Michaelis, Die Wasserstoffionenkonzentration, P. 42.

[30] Michaelis, Biochem. Zeit, **60,** 91 (1914)

[31] Michaelis and Rothstein, Biochem. Zeit. **110**, 217 (1920)

[32] Euler, Josephson and Myrbaeck, Zeit. Physiol. Chem. **134,** 39 (1923)

[33] Kuhn, Die Fermente und Ihre Wirkungen, Oppenheimer, Leipzig, 1924. P. 200.

[34] Kuhn, Die. Naturw. H. 35, 741 (1923)

[35] Clarke, Determination of Hydrogen Ions, 1924, was used as a source of information on the effective PH range of various buffers, together with reference to Sorenson, Biochem. Zeit. **21,** 131, 201 (1909)

[36] Sottery, Dissertation, Columbia University, 1925.

VITA

Chandler Dymock Ingersoll was born in Brooklyn, N. Y., on January 1, 1897. He was graduated from Peddie Institute, Hightstown, N. J., in 1914 and received the degree of B.S. with honors in chemistry from Columbia College in 1917. During the war he was in the United States Navy and held the successive ratings of seaman, chief gunner's mate, ensign, and Lieutenant (J.G.). From February, 1919 to September, 1923, he was associated in different capacities with the paint manufacturing business. In the fall of 1923 he returned to Columbia University and received his M.A. degree in chemistry the following spring. He was appointed a University Scholar for the year 1924-1925, during which time he was engaged in research under Professor J. M. Nelson. For the year 1925-1926 he has been appointed an American Field Service Fellow for work at the College de France. He is a member of Sigma Xi fraternity.

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